**Sequence Read Alignment**

* FASTQ files were aligned to the W3110 E. coli genome using BWA MEM (v0.7.17) and piped directly into samtools (v0.1.18)
  + bwa mem -t 4 -v 1 -T '30' -h '5' -M {EColi Genome} {FASTQ R1} {FASTQ R2} |samtools sort -@4 -O bam -o {Output BAM file}
* PCR duplicates were removed using Picard (v2.7.1)
  + picard MarkDuplicates INPUT={Input BAM} OUTPUT={Output BAM} METRICS\_FILE={Output Metrics} ASSUME\_SORTED='true' DUPLICATE\_SCORING\_STRATEGY='SUM\_OF\_BASE\_QUALITIES' READ\_NAME\_REGEX='[a-zA-Z0-9]+:[0-9]:([0-9]+):([0-9]+):([0-9]+).\*.' OPTICAL\_DUPLICATE\_PIXEL\_DISTANCE='100'
* Biological replicates were merged using samtools (v0.1.18)
  + samtools merge -@4 -o {Output BAM} {Rep1} {Rep2} …

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Filename** | **Replicate Sample ID** | | | |
| Fis\_30˚C\_RepMerge | 19780 | 20234 |  |  |
| Fis\_42˚C-6min\_RepMerge | 19786 | 20238 |  |  |
| RpoD\_30˚C\_RepMerge | 17253 | 17269 |  |  |
| RpoD\_42˚C-6min\_RepMerge | 17261 | 17276 |  |  |
| HimD\_30˚C\_RepMerge | 20219 | 20220 | 21320 |  |
| HimD\_42˚C-6min\_RepMerge | 20227 | 20228 | 21327 |  |
| MglB\_30˚C\_RepMerge | 17251 | 17267 |  |  |
| MglB\_42˚C-6min\_RepMerge | 17259 | 17274 |  |  |
| RpoH\_30˚C\_RepMerge | 17257 | 17273 | 21305 | 21306 |
| RpoH\_42˚C-6min\_RepMerge | 17265 | 17280 | 21313 | 21314 |
| RpoA\_30˚C\_RepMerge | 19784 | 20236 | 21299 |  |
| RpoA\_42˚C-6min\_RepMerge | 19790 | 20240 | 21307 |  |
| RpoB\_30˚C\_RepMerge | 17252 | 17268 | 21315 |  |
| RpoB\_42˚C-6min\_RepMerge | 17260 | 17275 | 21322 |  |
| RpoC\_30˚C\_RepMerge | 20223 | 20224 | 21316 | 21317 |
| RpoC\_42˚C-6min\_RepMerge | 20231 | 20232 | 21323 | 21324 |
| RpoN\_30˚C\_RepMerge | 20221 | 20222 |  |  |
| RpoN\_42˚C-6min\_RepMerge | 20229 | 20230 |  |  |

***de novo* motif discovery using MEME**

Figure 2A; 3D; 4C; 5C; 6A

* **Peak Calling using Genetrack**
* TAB files were generated using **BAM to scIDX** from ScriptManager v0.12.
* BAM Files: Fis\_30˚C\_RepMerge.bam, HimD\_30˚C\_RepMerge.bam, RpoD\_30˚C\_RepMerge.bam, RpoH\_30˚C\_RepMerge.bam, RpoN\_30˚C\_RepMerge.bam, Fis\_42˚C-6min\_RepMerge.bam, HimD\_42˚C-6min\_RepMerge.bam, RpoD\_42˚C-6min\_RepMerge.bam, RpoH\_42˚C-6min\_RepMerge.bam, RpoN\_42˚C-6min\_RepMerge.bam
  + Read 1
* genetrack.py script was run from command terminal using -s 5 -e 20 -F 1
* **Peak Calling using ChExMix**
* ChExMix v0.45 was run on command terminal for mock heat shock and heat shock datasets using MglB\_30˚C\_RepMerge.bam as control for mock heat shock and MglB\_42˚C-6min\_RepMerge.bam as control for heat shock data sets
  + java -Xmx24G -jar chexmix\_v0.45.jar --verbose --lenientplus --exptSAMPLE-1 <input BAM> --ctrl <control BAM> --format BAM --threads 4 --geninfo ec2.info.txt --seq ec2.fa --back ec2\_background\_model.txt --meme1proc --noread2 --scalewin 1000 --round 3 --minsubtypefrac 0.10 --minmodelupdateevents 50 –kldivergencethres -10 --motifpccthres 0.95 --prlogconf -4 --alphascale 1 --betascale 0.05 --epsilonscale 0.5 --mememinw 8 --mememaxw 21 --memenmotifs 3 --minroc 0.65 --minmodelupdaterefs 25 --pref -0.1 --numcomps 500 --win 250 --q 0.1 --minfold 1 --bmwindowmax 1000 --out <file\_name>
* Mock heat shock and heat shock Peak files for each protein (RpoD, RpoH, RpoN, Fis and HimD) were deduplicated, ranked and combined to obtain top 500 peaks for motif discovery using Combining Chexmix+Genetrack\_top500.R in R studio.
  + Manually combine mock heat shock and heat shock peaks for chexmix and genetrack datasets separately in excel
  + Using R studio, import these combined peak files and label them as myfile\_chex for chexmix and myfile\_genetrack for genetrack peaks. E.g myfile\_chex <- Fis\_chexmix\_combined, myfile\_genetrack <- Fis\_genetrack\_combined from Input peaks (Mock+Heat shock combined) subfolder in Peaks folder.
* The output top 500 peaks file was expanded to 100bp using **Expand BED File** from ScriptManager v0.12.
* BED Files: 2A (Top500\_RpoD\_100bp.bed), 3D (Top500\_RpoH\_100bp.bed), 4C (Top500\_RpoN\_100bp.bed), 5C (Top500\_fis\_100bp.bed), 6A (Top500\_HimD\_100bp.bed)
* The sequence was retrieved using **FASTA from BED** from ScriptManager v0.12.
  + Genome fasta: ec2.fa; force strandedness
* <https://meme-suite.org/meme/tools/meme> was used for *de novo* motif discovery using respective fasta files obtained from previous steps. Meme logo and motif PWM was downloaded from the website.

**NCIS Normalization**

* Scaling factor was calculated using **Calculate Scaling Factor** from ScriptManager v0.12.
* **Mock Heat Shock**
  + BAM Files: Fis\_30˚C\_RepMerge.bam, HimD\_30˚C\_RepMerge.bam, RpoA\_30˚C\_RepMerge.bam, RpoB\_30˚C\_RepMerge.bam, RpoC\_30˚C\_RepMerge.bam, RpoD\_30˚C\_RepMerge.bam, RpoH\_30˚C\_RepMerge.bam, RpoN\_30˚C\_RepMerge.bam
  + Control BAM: MglB\_30˚C\_RepMerge.bam
  + Window Size (bp) 500, Minimum Fraction 0.75
* **Heat Shock**
* BAM Files: Fis\_42˚C-6min\_RepMerge.bam, HimD\_42˚C-6min\_RepMerge.bam, RpoA\_42˚C-6min\_RepMerge.bam, RpoB\_42˚C-6min\_RepMerge.bam, RpoC\_42˚C-6min\_RepMerge.bam, RpoD\_42˚C-6min\_RepMerge.bam, RpoH\_42˚C-6min\_RepMerge.bam, RpoN\_42˚C-6min\_RepMerge.bam
* Control BAM: MglB\_42˚C-6min\_RepMerge.bam
  + Window Size (bp) 500, Minimum Fraction 0.75

**Heatmaps**

Figure 1A; S1; 3A; S3A; 4A; S4A

* Heatmaps were generated using **Tag Pileup** from ScriptManager v0.12.
  + BED File: 1A and S1 (BED-638\_Ref|ATG\_Sort|RpoD±80bp\_2000bp.bed), 3A and S3A (120-Nonaka\_Ref|ATG\_Sort|RpoH-30˚C±120 bp\_2000bp.bed), 4A and S4A (ATG\_Sort|RpoN-30˚C±500p\_2000bp.bed)
  + BAM Files: All bam files for Mock Heat shock datasets for 1A, All files for Mock+ Heat shock bam files for S1, Mock heat shock files (MglB, RpoD, RpoH, RpoN and RpoA) for 3A and 4A, Mock and Heat shock bam files for (MglB, RpoD, RpoH, RpoN and RpoA) for S3A and S4A
  + Read 1; Separate Strand; 6 bp tag shift, 2 bp bin size; sliding window 11
  + CDT files were scaled using **Scale Matrix Data** from ScriptManager v0.12
  + **Heat Map** from ScriptManager v0.12 was used to generate the figures
  + Starting Row: 1, Starting Column: 2; Select Color: custom (black); Image Height: 600, Image Width: 200; Contrast Threshold Absolute value: 1A and S1 (5 for Mock datasets and 2 for Heat shock), 3A and S3A (4 for Mock datasets and 2 for Heat sock) 4A and S4A (5 for Mock datasets and 1 for Heat shock) ; Image Compression: Treeview
  + **Merge Heatmaps** from ScriptManager v0.12 was used to merge sense and antisense png heatmaps

Figure 2B,C; S2A; 3E; S3C;4D; S4B; 5D; S5A; 6B; S6A

* Heatmaps were generated using **Tag Pileup** from ScriptManager v0.12.
* BED File: 2B,C and S2A(RpoD\_top196\_motifbound\_SORT+-50\_300bp.bed, RpoD\_1227\_motifbound\_SORT+-50\_2000bp.bed),3E and S3C (RpoH\_top52\_motifbound\_SORT+-50\_300bp.bed, RpoH\_1036\_motifbound\_SORT+-50\_2000bp.bed), 4D and S4B (RpoN\_top166\_motifbound\_SORT+-50\_300bp.bed, RpoN\_2323\_motifbound\_SORT+-50\_2000bp.bed), 5D and S5A (Fis\_1931\_motifbound\_SORT+-30\_300bp.bed, Fis\_1931\_motifbound\_SORT+-30\_2000bp.bed), 6B and S6B (HimD\_2293\_motifbound\_SORT+-40\_300bp.bed, HimD\_2293motifbound\_SORT+-40\_2000bp.bed)
* BAM files: All bam files for Mock Heat shock datasets for 2B, 3E, 4D, 5D, 6B and all Mock and Heat shock bam files for S2A, S3C, S4B, S5A, S6A. (For 2C, RpoD and RpoB Mock heat shock bam files)
  + Read 1; Separate Strand; 0 bp tag shift, 1 bp bin size for 300 bp bed and 2 bin size for 2000 bp bed;
  + CDT files were scaled using **Scale Matrix Data** from ScriptManager v0.12
  + **Heat Map** from ScriptManager v0.12 was used to generate the figures
  + Starting Row: 1, Starting Column: 2; Select Color: Blue for sense and red for antisense, Image Height: 600, Image Width: 200; Contrast Threshold Absolute value: 2B,C and S2A (10 for Mock datasets and 1 for Heat shock), 3E and S3C (10 for Mock datasets and 1 for Heat shock), 4D and S4B (12 for Mock datasets and 1 for Heat shock), 5D and S5A (10 for Mock datasets and 1 for Heat shock), 6B and S6A (10 for Mock datasets and 0.5 for Heat shock); Image Compression: Treeview
  + **Merge Heatmaps** from ScriptManager v0.12 was used to merge sense and antisense png heatmaps

**Composite plots**

Figure 1D; 3B; S3B; 4B; 5A,B

* Composite plots were generated using **Tag Pileup** from ScriptManager v0.12.
* BED file: 1D, 5A,B (BED-638\_Ref|ATG\_Sort|RpoD±80bp\_2000bp.bed), 3B, S3B (46\_heatshock\_1000bp.bed), 4B (ATG\_Sort|RpoN-30˚C±500p\_1000bp.bed)
* BAM: 1D (RpoD\_30˚C\_RepMerge.bam, RpoD\_42˚C-6min\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, MglB\_42˚C-6min\_RepMerge.bam, RpoA\_30˚C\_RepMerge.bam, RpoA\_42˚C-6min\_RepMerge.bam), 3B (RpoH\_30˚C\_RepMerge.bam, RpoH\_42˚C-6min\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, MglB\_42˚C-6min\_RepMerge.bam), S3B (RpoH\_30˚C\_RepMerge.bam, RpoH\_42˚C-6min\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, MglB\_42˚C-6min\_RepMerge.bam, RpoD\_30˚C\_RepMerge.bam, RpoD\_42˚C-6min\_RepMerge.bam, RpoN\_30˚C\_RepMerge.bam, RpoN\_42˚C-6min\_RepMerge.bam, RpoA\_30˚C\_RepMerge.bam, RpoA\_42˚C-6min\_RepMerge.bam), 4B (RpoN\_30˚C\_RepMerge.bam, RpoN\_42˚C-6min\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, MglB\_42˚C-6min\_RepMerge.bam, RpoD\_30˚C\_RepMerge.bam, RpoH\_30˚C\_RepMerge.bam, RpoA\_30˚C\_RepMerge.bam), 5A (Fis\_30˚C\_RepMerge.bam, Fis\_42˚C-6min\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, MglB\_42˚C-6min\_RepMerge.bam), 5B (HimD\_30˚C\_RepMerge.bam, HimD\_42˚C-6min\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, MglB\_42˚C-6min\_RepMerge.bam)
* Read 1; Separate Strand; 6 bp tag shift, 1 bp bin size for 1000 bp bed and 2 bin size for 2000 bp bed; sliding window 11, moving average 0.
* After NCIS normalization, average tag occupancy was plotted using Excel template for composite plot analysis and strands were merged.

Figure 2D; S2B; 3F, S3D, 4E; S4C, 5E; S5B; 6C; S6B

* Composite plots were generated using **Tag Pileup** from ScriptManager v0.12.
* BED files: 2D, S2B (RpoD\_top196\_motifbound\_SORT+-50\_2000bp.bed), 3F, S3D (RpoH\_top52motifbound\_SORT+-50\_2000bp.bed), 4E, S4C (RpoN\_top166\_motifbound\_SORT+-50\_2000bp.bed), 5E, S5B (Top200\_Fis\_motifbound\_SORT+-30\_2000bp.bed), 6C, S6B (Top200\_HimD\_motifbound\_SORT+-40\_2000bp.bed)
* BAM Files: All Mock heat shock bam files for 2D, 3F, 4E, 5E, 6C and all 18 bam files (mock + heat shock) for S2B, S3D, S4C, S5B, S6B
* Read 1; Separate Strand; 0 bp tag shift, 2 bp bin size; moving average 3
* After NCIS normalization, average tag occupancy was plotted using Excel template for composite plot analysis

**Averaged RNAP:RpoD occupancy ratio as a function RpoA promoter occupancy**

* CDT files were generated using **Tag Pileup** from ScriptManager v0.12.
* BED File: RpoD\_top196\_motifbound\_SORT+-50\_300bp.bed
* BAM Files: RpoA\_30˚C\_RepMerge.bam, RpoB\_30˚C\_RepMerge.bam, RpoC\_30˚C\_RepMerge.bam, RpoD\_30˚C\_RepMerge.bam
* Read 1; Separate Strand; 0 bp tag shift, 1 bp bin size
* CDT files were scaled using **Scale Matrix Data** from ScriptManager v0.12
* Using excel, RpoA, B, C, D occupancy levels were summed in the interval -80 to +10 on the RpoD motif/sense strand and from -10 to +80 on the opposite/antisense strand relative to the motif reference point. The top 70 RpoA-bound RpoD motifs (at 30˚C) were selected. All datasets were then sorted by RpoA occupancy level. RpoB:RpoD and RpoC:RpoD ratios were calculated and a 20 bin moving average (step size 1) calculated.

**Rank order by occupancy**

Figure 1B

* CDT files using **Tag Pileup** from ScriptManager v0.12 were generated.
* BED File: BED-638\_Ref|ATG\_Sort|RpoD±80bp-500+100.bed
* BAM: Fis\_30˚C\_RepMerge.bam, HimD\_30˚C\_RepMerge.bam, MglB\_30˚C\_RepMerge.bam, RpoA\_30˚C\_RepMerge.bam, RpoB\_30˚C\_RepMerge.bam, RpoC\_30˚C\_RepMerge.bam, RpoD\_30˚C\_RepMerge.bam, RpoH\_30˚C\_RepMerge.bam, RpoN\_30˚C\_RepMerge.bam
  + Read1; strands combined; 0 bp tag shift, 1 bp bin size
  + CDT files were scaled with respective scaling factors using **Scale Matrix Data** from ScriptManager v0.12
  + Sum of tags for each gene/operon was calculated using sum\_Row\_CDT.pl script
  + Using excel, log10 of each dataset was calculated, individually ranked in a descending order and plotted.

**Correlation analysis**

Figure 1C; 3C

* CDT files using **Tag Pileup** from ScriptManager v0.12 were generated.
* BED File: 1C (BED-638\_Ref|ATG\_Sort|RpoD±80bp-500+100.bed) 3C (46\_heatshock -500\_+100.bed)
  + BAM Files: All 18 (mock+heat shock) for 1C and 14 bam files (excluding fis and IHF mock+heat shock) for 3C
  + Read1; strands combined; 0 bp tag shift, 1 bp bin size
  + CDT files were scaled with respective scaling factors using **Scale Matrix Data** from ScriptManager v0.12
  + Sum of tags for each gene/operon was calculated using sum\_Row\_CDT.pl script
  + Tab file was manually created containing sum of tags for each gene/operon for the corresponding datasets and Pearson Correlation heatmap was generated using **correlation\_coeff\_v2.py**

**Four Color Plots**

Figure 2F; 3G; 4F; 5F; 6D

* Four color plots were generated by expanding the bed files to 100 bp using **Expand BED File** from ScriptManager v0.12.
  + BED Files: 2F (Top196\_RpoD\_sort+-50\_100bp.bed, bottom196\_RpoD\_sort+-50\_100bp.bed, RpoD\_1227\_motifbound\_SORT+-50\_100bp.bed), 3G (Top52\_RpoH\_100bp.bed, bottom52\_RpoH\_100bp.bed, RpoH\_1036\_motifbound\_SORT+-50\_100bp.bed), 4F (Top166\_RpoN\_100bp.bed, bottom166\_RpoN\_100bp.bed, RpoN\_2323\_motifbound\_SORT+-50\_100bp.bed), 5F (Top200\_Fis\_100bp.bed, Bottom 200\_Fis\_100bp.bed, Fis\_1931\_motifbound\_SORT+-30\_100bp.bed), 6D (Top200\_HimD\_100bp.bed, bottom200\_HimD\_100bp.bed, HimD\_2293\_motifbound\_SORT+-40\_100bp.bed)
* The sequence was retrieved using **FASTA from BED** from ScriptManager v0.12.
  + Genome fasta: ec2.fa; force strandedness
* The plot was generated using **4Color Sequence Plot** from ScriptManager v0.12.
  + A: Red, T: Green, G: Yellow, C: Blue
* Meme motif analysis was performed by running meme from command terminal using fasta files from previous step corresponding to the respective panel
* <input fasta file> -dna -nostatus -time 14400 -mod zoops -nmotifs 1 -minw 100 - maxw 100
* -time can be increased or decreased depending upon the number of DNA sequences being analyzed

**DNA shape analysis**

Figure 2G; 3H; 4G; 5G; 6E

* For DNA shape analysis, sorted bed files were expanded to 60 bp using **Expand BED File** and used as input for **DNA Shape from BED** from ScriptManager v0.12.
  + BED files: 2G (top100\_RpoD\_motifbound\_SORT+-50\_60bp.bed, bottom100\_RpoD\_motifbound\_SORT+-50\_60bp.bed), 3H (top52\_RpoH\_motifbound\_SORT+-50\_60bp.bed, bottom52\_RpoH\_motifbound\_SORT+-50\_60bp.bed), 4G (top100\_RpoN\_motifbound\_SORT+-50\_60bp.bed, bottom100\_RpoN\_motifbound\_SORT+-50\_60bp.bed), 5G (top100\_Fis\_motifbound\_SORT+-30\_60bp.bed, bottom100\_Fis\_motifbound\_SORT+-30\_60bp.bed), 6E (top100\_HimD\_motifbound\_SORT+-40\_60bp.bed, bottom100\_HimD\_motifbound\_SORT+-40\_60bp.bed)
  + Genome fasta: ec2.fa; force strandedness
* The DNA shape values for the “Top 100” and “Bottom 100” (“Top 52” and “Bottom 52” for RPoH) motif binding sites were rank ordered in Excel (=RANK.AVG(cell, range)), then a Mann-Whitney *U* test was performed on the ranks of the two groups.
  + U1 = sum of ranks of the “Top 100 ” sites and “Top 52” for RpoH
  + U2 = sum of ranks of the “Bottom 100 ” sites and “Bottom 52” for RpoH
  + U = U1 +U2
  + n1 = 100, n2 = 100 (For RpoH: n1= 52, n2 = 52)
  + mean of ranks = U/2
  + Standard deviation =SQRT(n1\*n2\*(n1+n2+1)/12)
  + Z-score =(U1-(mean of ranks))/(Standard Deviation)
* If |Z| > 2, then the position was considered to have a significant DNA shape difference between the two groups of sites.

**Structure visualization**

Figure 5H; 6F

* Crystal structures visualized with Pymol